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EVIDENCE THAT THE CONTRACTILE RESPONSE OF THE GUINEA-PIG ILEUM TO CAPSAICIN IS DUE TO RELEASE OF SUBSTANCE P

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SUMMARY

1. The possible roles of substance P and opioids in the contractile response of the isolated guinea-pig ileum to the sensory stimulant drug capsaicin were investigated, and the contractions were found to be inhibited by about 60% in preparations desensitized to substance P.

2. Contractions evoked by stimulation of the mesenteric nerves in the presence of the adrenergic blocking drug guanethidine were inhibited by about 75% after the ileum had been rendered insensitive to substance P.

3. Atropine partially inhibited the effect of capsaicin. The atropine-resistant component of the contractile response to capsaicin was inhibited by more than 85% in preparations desensitized to substance P and almost abolished by the substance P antagonist, (D-Pro^2,D-Trp^{7,9})-substance P.

4. The opioid peptide (D-Met^2,Pro^5)-enkephalinamide inhibited, whereas the opiate antagonist naloxone enhanced the atropine-resistant contractions in response to capsaicin.

5. The results indicate that the contractile response of the guinea-pig ileum to capsaicin and mesenteric nerve stimulation is mediated by release of substance P, presumably from sensory nerve endings in the gut. Substance P appears to act on the smooth muscle both directly and indirectly via cholinergic neurones. It is proposed that opioids modulate the non-cholinergic response to capsaicin by inhibiting the release of substance P.

INTRODUCTION

The sensory stimulant drug capsaicin has been shown to release substance P from central and peripheral endings of sensory neurones (Gamse, Molnar & Lembeck, 1979; Theriault, Otsuka & Jessell, 1979; Yaksh, Jessell, Gamse, Mudge & Leeman, 1980; Gamse, Wax, Zigmond & Leeman, 1981). Treatment of animals with high doses of

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capsaicin causes functional impairment of and depletion of substance P from these neurones (Jancsó, Jancsó-Gábor & Szolcsányi, 1967; Jessell, Iversen & Cuello, 1978; Gamse, Holzer & Lembeck, 1980; Nagy, Vincent, Staines, Fibiger, Reisine & Yamamura, 1980; Cuello, Gamse, Holzer & Lembeck, 1981; Gamse *et al.* 1981). Antidromic electrical stimulation of capsaicin-sensitive sensory neurones gives rise to peripheral vasodilatation (see Lembeck & Holzer, 1979). Recently, evidence has been presented that the mediator of this local efferent response is substance P (Lembeck & Holzer, 1979; Rosell, Olgart, Gazelius, Panopoulos, Folkers & Hörig, 1981; Lembeck, Folkers & Donnerer, 1981).

In the guinea-pig ileum capsaicin has been shown to cause neurally mediated contractions, followed by specific and irreversible desensitization. Detailed analysis has indicated that both extrinsic non-parasympathetic and intrinsic cholinergic neurones are involved in this action of capsaicin. Contractions to capsaicin were strongly inhibited, but never abolished by the cholinergic antagonist hyoscine (Barthó & Szolcsányi, 1978). Following blockade of peripheral noradrenergic neurones by guanethidine, stimulation of mesenteric nerves has been shown to cause cholinergic contractions of the guinea-pig ileum. As this type of response was also irreversibly inhibited by desensitization to capsaicin it was concluded that the contractile responses to capsaicin and to mesenteric nerve stimulation have similar mechanisms, and the participation of extrinsic sensory neurones was proposed (Szolcsányi & Barthó, 1978). The mode of activation of intestinal cholinergic neurones remained unknown.

Neurally mediated, capsaicin-sensitive excitatory responses have also been demonstrated in other segments of the guinea-pig intestinal tract (Szolcsányi & Barthó, 1979; Barthó & Szolcsányi, 1981*a*) as well as in the rabbit small intestine (Barthó & Szolcsányi, 1980).

Substance P-containing neurones in the intestine are of both intrinsic and extrinsic origin (Costa, Cuello, Furness & Franco, 1980; Costa, Furness, Llewellyn-Smith & Cuello, 1981) and substance P has been proposed as a neurotransmitter within the enteric plexuses (Katayama, North & Williams, 1979). There is now good evidence that neurally mediated non-cholinergic contractions of the guinea-pig ileum are mediated by the release of substance P or a related substance (Franco, Costa & Furness, 1979; Monier & Kitabgi, 1980; Gintzler, 1980; Hutchinson & Dockray, 1981). Substance P is capable of contracting the intestinal smooth muscle both directly and indirectly via release of acetylcholine (Holzer & Lembeck, 1980; Holzer, Emson, Iversen & Sharman, 1981).

The present experiments were carried out to investigate the involvement of substance P in the contractile response to capsaicin and mesenteric nerve stimulation in the guinea-pig ileum. In addition, the question was investigated of whether exogenously applied or endogenously released opioids modulate the activity of neurones involved in the contractile response to capsaicin.

METHODS

Experimental procedures

Segments of guinea-pig ileum were suspended in Tyrode solution, gassed with a mixture of 95% O_2 and 5% CO_2, at a temperature of 37 °C. Two different experimental arrangements were used, one for auxotonic and one for isotonic recordings of contractions. For auxotonic recordings, intestinal segments of approximately 4 cm length were connected to a lever displacement measuring system (Hugo Sachs Elektronik), supplemented by a fine spring; contractions were recorded on a Watanabe pen recorder. The resting tension applied to the tissue was 0·5 g. This arrangement was used in experiments involving desensitization to substance P in the absence of atropine while the desensitizing concentration of substance P was continually present in the bath. Under isotonic conditions, in the absence of atropine, ileal segments did not relax completely in the continuous presence of a desensitizing concentration of substance P; this is apparently due to the activation of cholinergic neurones by this peptide (Holzer & Lembeck, 1980; Holzer *et al.* 1981). Under isometric (Franco *et al.* 1979; Hutchinson & Dockray, 1981) and auxotonic conditions, however, the contractile response to substance P fades away completely within a few minutes, even in the absence of a cholinergic antagonist. In the present experiments, the ileum was desensitized to substance P by exposing it to 75 nM-substance P (Franco *et al.* 1979). Ten minutes later, when the initial contraction had waned completely, the drugs to be tested were added in the continuous presence of the desensitizing concentration of substance P. When the same dose of substance P was applied again, the contractile response was only 21 ± 4% of that on first administration ($n = 5$), while responses to histamine (0·1–0·3 μM) or nicotine (6 μM) were not reduced ($n = 5$), indicating specific desensitization against substance P.

In some experiments ileal segments were set up together with their mesenteric supplies. Periarterial stimulation of the mesenteric nerves was performed as described earlier (Szolcsányi & Barthó, 1978). These experiments were carried out in the presence of guanethidine (3·4 μM); the animals from which the intestinal segments were obtained had also been treated with guanethidine (10 mg guanethidine sulphate/kg intraperitoneally, 1 h before they were killed). Relaxation of the ileum in response to mesenteric nerve stimulation was never seen, indicating a complete functional blockade of sympathetic adrenergic neurones.

In another set of experiments transmural field stimulation was applied through a pair of longitudinal platinum electrodes. Single pulses of 0·1 ms width (supramaximal voltage) were delivered at a frequency of 0·1 Hz.

For isotonic recordings, ileal segments of approximately 1 cm length were mounted under a resting load of 0·3 g and contractions recorded isotonically. Unless indicated otherwise, these experiments were performed in the presence of atropine (2 μM). Substance P desensitization was induced in the presence of atropine in the same way as has been described above. In agreement with earlier data (Holzer & Lembeck, 1980; Holzer *et al.* 1981) the contractile effect of substance P (75 nM) invariably faded away within 5–6 min following the addition of the peptide, Here again, substance P desensitization did not reduce histamine-induced contractions of the ileum ($n = 5$).

Drugs and chemicals

The following drugs were used: atropine sulphate (Merck), capsaicin (Merck), (D-Met^2,Pro^5)-enkephalinamide (Research Institute for Pharmaceutical Chemistry, Budapest), guanethidine sulphate (Ciba), histamine dihydrochloride (Knoll), nicotine bitartrate (Sigma), substance P (Peninsula), (D-Pro^2,D-Trp^{7,9})-substance P (Ferring), tetrodotoxin (Sankyo). A stock solution of capsaicin (33 mM) was prepared with absolute ethanol; dilutions were made with 0·15 M-NaCl solution. Stock solutions of substance P (0·75 mM) or (D-Pro^2,D-Trp^{7,9})-substance P (6·5 mM) were prepared with 0·01 M-acetic acid and dilutions made with NaCl solution containing gelatine (1 g/l) to avoid adsorption to surfaces. All other drugs were made up in 0·15 M-NaCl. Applied in volumes used for drug administration, the respective solvents had no effect on the ileum.

Statistics

Quantitative data are presented as mean $\pm$ S.E. of means throughout the paper. The heights of contractions are expressed as percentages of the maximal contraction (% max) evoked by histamine (10 μM). Significant differences between means were assessed using Student's t test for unpaired (capsaicin) or paired data (mesenteric nerve stimulation).

RESULTS

As reported earlier (Barthó & Szolcsányi, 1978), capsaicin (0·1–3·3 μM, contact time 1 min) caused concentration-dependent contractions of the guinea-pig ileum. The response to 3·3 μM-capsaicin, recorded auxotonically, was 44·9 ± 2·2 % max. A second exposure to 3·3 μM-capsaicin, 15–30 min after the first, failed to produce a contraction indicating desensitization of the guinea-pig ileum to capsaicin (Fig. 1). The smallest concentration of capsaicin applied (0·1 μM) caused no apparent desensitization. Therefore, ileal segments were exposed only once to concentrations of capsaicin above 0·1 μM in the following experiments.

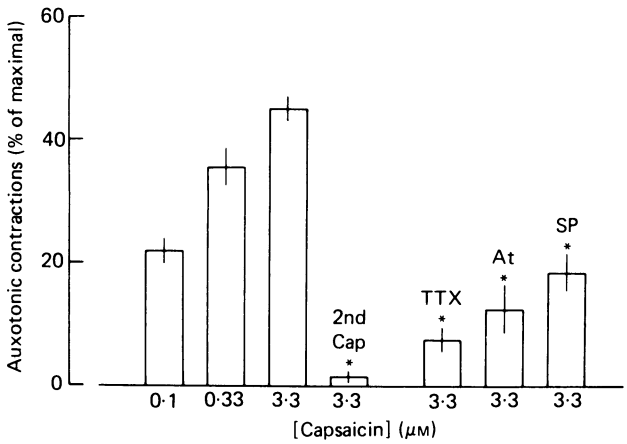

Fig. 1. Effect of tetrodotoxin (TTX, 1 μM, $n = 6$), atropine (At, 2 μM, $n = 7$) and substance P desensitization (SP, 75 nM, $n = 7$) on the contractile responses of the guinea-pig ileum to capsaicin (3·3 μM). The first three columns show control responses to capsaicin ($n = 9$–12). Note the strong desensitization to capsaicin as indicated by the small response to a second exposure to 3·3 μM-capsaicin (2nd Cap, $n = 6$). Contractions were recorded auxotonically and are expressed in % of the maximal contraction evoked by histamine (10 μM). Asterisks indicate significant differences ($P < 0·05$ or less) from the control responses (third column). Means ± S.E. of means.

Contractions elicited by capsaicin (3·3 μM) were substantially reduced by tetrodotoxin (1 μM), a drug that specifically blocks Na influx-mediated nerve conduction. Similarly, blockade of cholinergic muscarinic receptors by atropine (2 μM) strongly inhibited, but did not abolish contractions induced by capsaicin (Fig. 1). The contact time for both tetrodotoxin and atropine was 10 min. As compared with the control group, atropine reduced the contractile responses to capsaicin by 72 %. Similar degrees of inhibition were found when hyoscine was used as the cholinergic antagonist (Barthó & Szolcsányi, 1978). Contractile responses, resistant to the concentration of atropine used in the present experiments (2 μM) were regarded as non-cholinergic in nature, since a ten times lower concentration of atropine totally abolished cholinergic 'twitch' contractions, evoked by field stimulation of intramural cholinergic neurones of the intestine ($n = 4$).

Desensitization of the ileum to substance P inhibited the contractile effect of capsaicin (3·3 μM) by about 60 % (Fig. 1).

Stimulation of the mesenteric periarterial nerves in the presence of guanethidine caused frequency-dependent contractions of the ileum. These responses were reproducible over at least four consecutive cycles of stimulation (5, 10 and 20 Hz each). Substance P desensitization strongly inhibited contractions of the guinea-pig ileum evoked by mesenteric nerve stimulation (Fig. 2).

Isotonic contractions evoked by capsaicin (3·3 μM) in the absence of atropine were greater than those obtained under auxotonic conditions (62·3 $\pm$ 2·4 *vs.* 44·9 $\pm$ 2·2 % max, $n = 6$ and 9, respectively). In order to study the non-cholinergic component

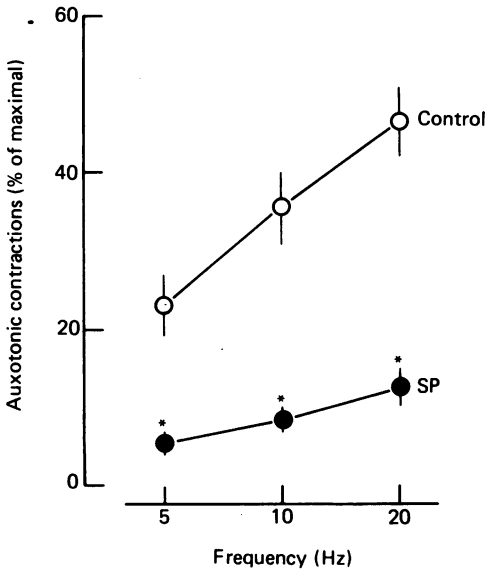

Fig. 2. Auxotonic contractions of the guinea-pig ileum upon mesenteric nerve stimulation (10 V, 0·5 ms, 20 s trains at frequencies of 5–20 Hz) before (control) and after exposure for 10 min to a desensitizing concentration of substance P (SP, 75 nM, $n = 5$). Ileal segments were obtained from guinea-pigs treated with guanethidine sulphate 10 mg/kg 1 h before they were killed; guanethidine (3·4 μM) was also present throughout the experiments. Asterisks indicate statistically significant inhibitions ($P < 0·05$ or less). Means $\pm$ S.E. of means.

of the contractile response to capsaicin the intestinal segments were exposed to atropine (2 μM) for 40–60 min before the addition of capsaicin. This was done because preliminary experiments had shown that the continuous presence of cholinergic antagonists reduced the variability in the contractile responses to capsaicin. Then, still in the presence of atropine, a test concentration of capsaicin (0·1 μM) was added for 40 s. Intestinal segments were regarded as unresponsive to capsaicin and immediately discarded when the response to this test concentration was less than 20 % max. This was the case in twenty-one out of the 102 preparations tested.

In the presence of atropine, contractions evoked by capsaicin (3·3 μM) still reached 42·1 $\pm$ 1·8 % max ($n = 12$, Fig. 3). Here again, a second application was ineffective, indicating complete desensitization of the guinea-pig ileum to capsaicin. The effects of histamine or substance P were not altered in ileal segments rendered insensitive to capsaicin ($n = 5$). Exposure to tetrodotoxin (1 μM) for 10 min significantly

inhibited the atropine-resistant effect of capsaicin. However, the results were extremely variable and the mean inhibition was less than 50% (Fig. 3).

The atropine-resistant effect of capsaicin was reduced by more than 85% ($n = 6$) in preparations which had been desensitized to substance P. Moreover, an antagonist of substance P, (D-Pro^2,D-Trp^{7,9})-substance P (0·13 mM, contact time 5 min), abolished the atropine-resistant responses to capsaicin ($n = 5$, Fig. 3). Half-maximal responses to substance P were also abolished by this concentration of (D-Pro^2,D-

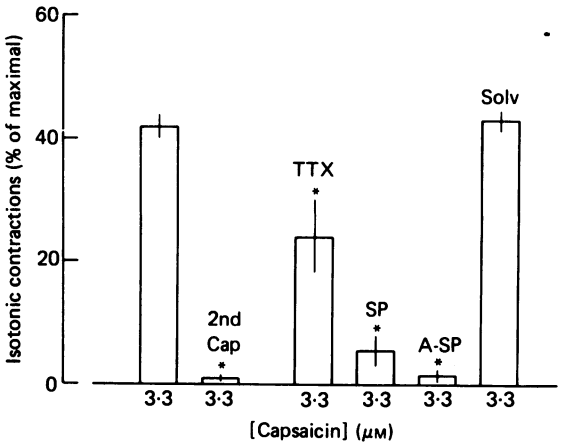

Fig. 3. Effect of tetrodotoxin (TTX, 1 μM, $n = 6$), substance P desensitization (SP, 75 nM, $n = 6$), the substance P antagonist (D-Pro^2,D-Trp^{7,9})-substance P (A-SP, 0·13 mM, $n = 5$) and its solvent (Solv, $n = 4$), as well as capsaicin desensitization (2nd Cap, $n = 5$) on the contractile action of 3·3 μM-capsaicin on the guinea-pig ileum. Contractions were recorded isotonically in the presence of atropine (2 μM) and are expressed as % of the maximal contraction evoked by 10 μM-histamine. Asterisks indicate significant differences from the control responses (first column, $n = 12$). Means ± S.E. of means.

Trp^{7,9})-substance P (0·13 mM), while those to histamine remained unaltered. On first exposure the substance P antagonist exerted a transient contractile effect on the guinea-pig ileum (see Leander, Håkanson, Rosell, Folkers, Sundler & Tornqvist, 1981). Therefore, the ileum was incubated with a smaller concentration of the antagonist (17–34 μM) for 10 min, which prevented the appearance of a contraction during the subsequent exposure to the higher concentration (0·13 mM). The substance P-antagonizing effect of (D-Pro^2,D-Trp^{7,9})-substance P was transient and the ileum regained its original sensitivity to substance P within 10–15 min after washing out the antagonist. Addition of the solvent only (0·01 M-acetic acid) did not influence the contractile response to capsaicin (Fig. 3).

The synthetic enkephalin analogue (Székely, Rónai, Dunai-Kovács, Miglécz, Berzétri, Bajusz & Gráf, 1977), (D-Met^2,Pro^5)-enkephalinamide (1 μM, contact time 10 min), reduced the atropine-resistant response to capsaicin (Fig. 4*A*), but did not inhibit contractions evoked by substance P or histamine ($n = 5$). Conversely, the specific opiate receptor blocking drug naloxone (1 μM, contact time 10 min) significantly enhanced the atropine-resistant responses to capsaicin (0·33–3·3 μM, Fig. 4*A*). Naloxone alone had no effect on most preparations, while in a few ileal segments

it caused a small and transient contraction. In the presence of naloxone (1 μM), atropine-resistant contractions evoked by capsaicin (3·3 μM) were inhibited by capsaicin desensitization, by tetrodotoxin or by substance P desensitization (Fig. 4*B*) to similar degrees as in the absence of naloxone (compare Figs. 3 and 4*B*). Here again, the results were most variable in the presence of tetrodotoxin.

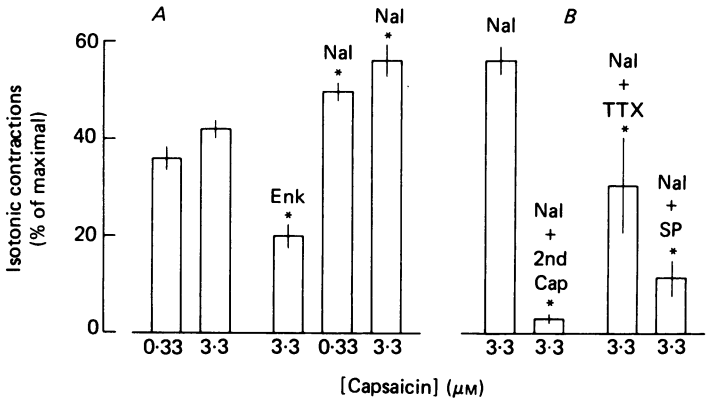

Fig. 4. Isotonic contractions of the guinea-pig ileum in the presence of atropine (2 μM), expressed as % of the maximal contraction evoked by histamine (10 μM). *A*, effect of (D-Met^2,Pro^5)-enkephalinamide (Enk, 1 μM, $n = 6$) and naloxone (Nal, 1 μM, $n = 9$ each) on the contractile action of capsaicin (0·33–3·3 μM) in the presence of atropine. Asterisks indicate significant differences from the respective control responses (first two columns, $n = 10$–12). Means ± S.E. of means. *B*, effect of capsaicin desensitization (Nal + 2nd Cap, $n = 6$), tetrodotoxin (Nal + TTX, $n = 5$), and substance P desensitization (Nal + SP; $n = 9$) on the contractile action of capsaicin (3·3 μM) in the presence of naloxone (Nal) and atropine. The concentrations used were: naloxone 1 μM, tetrodotoxin 1 μM, SP 75 nM. Asterisks indicate significant differences from the control responses (first column, $n = 9$). Means ± S.E. of means.

DISCUSSION

Previous work on the contractile action of capsaicin on the guinea-pig ileum has revealed that the effect of capsaicin is mediated to a considerable degree by the release of acetylcholine (Barthó & Szolcsányi, 1978). The present findings indicate that release of substance P is also involved, since both desensitization of the ileum to substance P and an antagonist of substance P inhibited the response to capsaicin. While neither atropine nor desensitization to substance P alone were capable of abolishing the effect of capsaicin (see Fig. 1), the response remaining in the presence of atropine was almost completely suppressed by rendering the ileum insensitive to substance P as well as by the substance P antagonist (see Fig. 2). Recently, evidence has accumulated that neurally mediated atropine-resistant contractions of the intestine elicited by electrical stimulation (Franco *et al.* 1979; Leander *et al.* 1981), neurotensin (Monier & Kitabgi, 1980), cholecystokinin octapeptide (Hutchinson & Dockray, 1981), and naloxone (Gintzler, 1980) are also mediated by the release of substance P. In these instances it seems likely that the final mediator of the response, acting on the smooth muscle, is substance P or a related substance. This seems to apply for the non-cholinergic component of the contractile effect of capsaicin as well.

The mechanism of the action of capsaicin, however, is not fully understood. On the grounds detailed below, we propose that the primary action of capsaicin is release of substance P, which both directly and indirectly, via activation of cholinergic neurones, causes contraction of the guinea-pig ileum.

There is good evidence that capsaicin acts selectively on a population of sensory neurones, a considerable portion of which contains and releases substance P (see Introduction). Most of the substance P-containing neurones in the gut seem to be of intrinsic origin (Costa *et al.* 1980, 1981; Jessen, Saffrey, van Noorden, Bloom, Polak & Burnstock, 1980), and systemic treatment of rats and guinea-pigs with capsaicin does not change the content of radioimmunoassayable substance P in the intestine (Holzer, Gamse & Lembeck, 1980; Gamse *et al.* 1981; Buck, Desmukh, Yamamura & Burks, 1981). Immunohistochemical investigations have, however, shown that the gut is also innervated by extrinsic, probably sensory, substance P fibres which run in the mesenteric nerves (Costa *et al.* 1980, 1981). More recently Furness, Papka, Della, Costa & Eskay (1982) have demonstrated that these extrinsic fibres are depleted of substance P by systemic treatment with capsaicin. As it has been shown that extrinsic non-parasympathetic neurones, running in the mesenteric nerves, play a major role in the action of capsaicin on the guinea-pig ileum (Barthó & Szolcsányi, 1978; Szolcsányi & Barthó, 1978) it may be inferred that the contractile effect of capsaicin is brought about by the release of substance P from sensory fibres terminating in the gut. Contractile responses to electrical stimulation of substance P neurones intrinsic to the intestine are apparently not affected by desensitization of the guinea-pig ileum to capsaicin (Barthó, Sebök & Szolcsányi, 1982).

Contractions evoked by electrical stimulation of intrinsic cholinergic neurones are not affected by rendering the ileum insensitive to capsaicin (Szolcsányi & Barthó, 1978). Thus it is likely that capsaicin does not directly stimulate cholinergic neurones to release acetylcholine, but acts on them indirectly via release of substance P. Substance P has been shown to excite myenteric neurones in the guinea-pig ileum (Katayama *et al.* 1979), and analysis of the contractile action of substance P in the guinea-pig ileum has revealed activation of cholinergic neurones by this peptide (Holzer & Lembeck, 1980; Holzer *et al.* 1981). It appears likely, therefore, that substance P released by capsaicin acts on cholinergic neurones of the myenteric plexus. The non-cholinergic component in the contractile effect of capsaicin is in part inhibited by tetrodotoxin. The tetrodotoxin-insensitive component can probably be explained by diffusion of substance P, released from capsaicin-sensitive nerve endings, to the longitudinal muscle; the release of substance P by capsaicin from the central endings of sensory neurones is not affected by tetrodotoxin (Gamse *et al.* 1979). The tetrodotoxin-sensitive component is apparently also mediated by substance P; a possible explanation seems that intrinsic substance P neurones are also activated in the course of the action of capsaicin. Another explanation might be that capsaicin excites nerve endings as well as axons of capsaicin-sensitive neurones, since a desensitizing action of capsaicin on axons of capsaicin-sensitive nerves has been described (Jancsó, Király & Jancsó-Gábor, 1980; Gamse, Petsche, Lembeck & Jancsó, 1982).

The discrepancy between the study of Barthó & Szolcsányi (1978), who found no evidence for the involvement of substance P in the contractile effect of capsaicin, and

the present study may be due to differences in the experimental protocol. In the former study the desensitizing concentration of substance P was washed out before capsaicin was added, but in this study capsaicin was tested while substance P was still present in the bath. As the substance P receptors located on intramural cholinergic neurones are apparently less readily desensitized by substance P than those located on the smooth muscle (Holzer & Lembeck, 1980; Holzer *et al.* 1981) it might also be argued that in the experiments of Barthó & Szolcsányi (1978) desensitization of the neural substance P receptors was not sufficient to prevent the activation of cholinergic and possibly other intestinal neurones.

The findings that the opiate agonist, (D-Met^2,Pro^5)-enkephalinamide inhibited, while the opiate antagonist naloxone enhanced the non-cholinergic response to capsaicin indicate that endogenous opioid substances modulate this response. Neurones containing the opioid peptides, enkephalins and endorphins, occur in the gut (Hughes, Kosterlitz & Smith, 1977; Jessen *et al.* 1980; Schultzberg, Hökfelt, Nilsson, Terenius, Rehfeld, Brown, Elde, Goldstein & Said, 1980) and there is direct evidence that enkephalins are released during electrical stimulation (Corbett, McKnight & Kosterlitz, 1981). Non-cholinergic, presumably substance P-mediated, contractile responses of the guinea-pig ileum to neurotensin (Monier & Kitabgi, 1981) or to field stimulation (Barthó *et al.* 1982; Gintzler & Scalisi, 1982) are also inhibited by opiate agonists and the latter response is also enhanced by naloxone (Barthó *et al.* 1982; Gintzler & Scalisi, 1982). Moreover, opiates seem to inhibit the release of substance P from the central (Jessell & Iversen, 1977; Yaksh *et al.* 1980) and, as indicated by circumstantial evidence, from the peripheral endings of capsaicin-sensitive sensory neurones as well (Barthó & Szolcsányi, 1981*b*). Thus it would appear that the inhibitory action of the enkephalin analogue on the atropine-resistant effect of capsaicin is due to inhibition of the release of substance P from nerve endings in the gut, which is all the more likely since neither the enkephalin analogue used nor naloxone (Chipkin, Stewart & Morris, 1978) affect the contractile responses of the guinea-pig ileum to exogenous substance P.

Desensitization of the guinea-pig ileum to substance P not only inhibited the contractile response to capsaicin, but also that to mesenteric nerve stimulation. In both responses, extrinsic, non-parasympathetic capsaicin-sensitive neurones seem to play a major role (Barthó & Szolcsányi, 1978; Szolcsányi & Barthó, 1978), but cholinergic neurones are also involved. It has been shown that contractions due to mesenteric nerve stimulation are more strongly inhibited by blockade of muscarinic acetylcholine receptors than contractions evoked by capsaicin (Szolcsányi & Barthó, 1978). Since desensitization to substance P also very strongly inhibited the response to mesenteric nerve stimulation, it may be inferred that substance P also mediates this type of cholinergic contraction. The fact that the responses to both capsaicin and mesenteric nerve stimulation are inhibited by rendering the guinea-pig ileum insensitive to substance P confirms the hypothesis of Szolcsányi & Barthó (1978) that the non-cholinergic neurones in the guinea-pig ileum, which are excited by capsaicin, correspond to those excited by mesenteric nerve stimulation and suggest that they are identical to sensory substance P-containing neurones.

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